Studies on Enzyme Action.—Lipase.

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It was first shown by J. Reynolds Green, in 1889, that germinating seeds of the castor-oil plant contain an enzyme which is capable of hydrolysing castor oil. Of late years an industry has been based on this discovery, as it has been found that if ground ungerminated castor-oil seed be mixed with an ordinary fatty oil and a small quantity of acid (preferably acetic), the fatty matter is almost entirely hydrolysed into glycerol and fatty acid: as the change takes place rapidly, at a little above an ordinary temperature, the hydrolysis of fats may be effected in this manner with considerable advantage on economic as well as other grounds.*

The study of vegetable lipase is of special importance, as the ordinary fats —which are hydrolysed under its influence with peculiar readiness—are not asymmetric material but simply glycerides of acids of the acetic or oleic series. The discovery of the nature of the process is, therefore, of particular interest, in order that a comparison may be instituted between this enzyme and those which are known to act selectively. The interest of the inquiry is enhanced by the fact that animal lipase, according to Dakin,† acts selectively on the mixture of ethereal salts derived from inactive mandelic acid, hydrolysing the dextro-constituent more rapidly than the lævo-constituent; but even in this case, inasmuch as the whole of the ethereal salt is hydrolysed eventually, the selective effect of lipase is of a different order from that displayed, for example, by an enzyme of the sucroclastic class, which can only attack one member of a pair of enantiomorphous isomerides.

Among the chief points of interest already established by Connstein and his fellow workers are, firstly, that Ricinus lipase is effective only in presence of acid; secondly, that it acts preferentially on the natural fats; other ethereal salts are scarcely if at all attacked by it.‡

* 'Comp. Connstein: V. Internationaler Congress für Angewandte Chemie,' Berlin, June, 1903, vol. 2, p. 537.

† 'Journal of Physiology,' 1903, vol. 30, p. 253; 1905, vol. 32, p. 199.

‡ The literature of the subject is summarised in an article by Connstein, "Ueber fermentative Fettspaltung," in Asher and Spiro's 'Ergebnisse der Physiologie, Biochemie,' 1904, vol. 3, p. 194.

Animal lipase, however, from the liver or pancreas of the pig, according to Kastle and Loevenhart,* manifests considerable activity in hydrolysing simple ethereal salts, the action being greater in the case of the higher than of the lower terms of the series, as shown by the fact that ethylic butyrate is more readily hydrolysed than is ethylic acetate. Dakin's observations may be regarded as confirming this conclusion. Whether animal lipase acts on natural fats is not yet satisfactorily determined. Lewkowitsch states that he could not carry the hydrolysis of cotton-seed oil beyond 3 per cent.; he is inclined to attribute this want of success, however, to the fact that he could not secure a satisfactory emulsion.

The activity of animal lipase, it should be mentioned, is said by Magnus† to be conditioned by two substances, one of which is dialysable and not destroyed by boiling whilst the other is destroyed by heat and not dialysable.

Judging from my own experiments, it is clear that the investigation of lipase presents peculiar difficulties. It would scarcely be worth while to put the results on record, were it not desirable to call attention to issues which they raise.

The material used was simply ground castor-oil seed, free from husk, when the action of the enzyme on castor oil was under consideration; whilst for the purpose of studying the action of the enzyme on other ethereal salts, this material was carefully freed from oil by washing it with ether and dried by exposure to the air on a porous plate. In nearly every case, toluene was added to maintain sterile conditions.

Connstein's contention has been confirmed that the presence of acid is necessary to condition the hydrolysis and that practically any acid is effective, provided a sufficient amount be used. Aspartic and glutamic acids—which are formed at an early stage of the germination of seeds—were found to be highly active; glycin and asparagin, however, were practically without effect. Thus, in an experiment in which a mixture of 5 c.c. of olive oil, 1 gramme fat-free castor-oil seed and 10 c.c. 3/100 N sulphuric acid was digested at 38° during 18 hours, the amount of oleic acid liberated was 4·145 grammes. In a blank experiment from which the enzyme was omitted, the amount of oleic acid liberated at the end of 18 hours was 0·1087 gramme; at the end of 24 hours 0·1128 gramme; and at the end of 48 hours 0·132 gramme. On digesting 5 grammes of castor-oil seed paste at 38° with 5 c.c. of a 1-per-cent. solution of aspartic acid, 4 c.c. of water and 1 c.c. of toluene, the amount of ricinoleic acid liberated at the end of 19 hours was 2·99 grammes. In a similar experiment with glutamic acid, the amount liberated was

* 'American Chemical Journal,' 1900, vol. 24, p. 491.

† 'Zeit. Physiol. Chem.,' 1904, vol. 42, p. 149.

3·024 grammes. On digesting a mixture of 5 grammes of the seed paste, free from husk, with 9 c.c. of water and 1 c.c. of toluene at 38°, the amount of ricinoleic acid liberated after 19 hours was 0·109 gramme; after 116 hours 0·387 gramme; and after 164 hours 0·596 gramme. When chloral hydrate is used as antiseptic, after a time a sudden great increase in the amount of acid liberated is observed; probably this is conditioned by the formation of mineral acid by decomposition of the chloral hydrate.

As acids do not act equally when used in equivalent quantities, although when used in sufficient amount weak acids are as effective as strong acids, it is probable that the strength of the acid is a factor in the action.

All attempts resulted in failure which were made to obtain an extract containing an enzyme, whether from the freshly-ground material directly or after this had been deprived of the fatty matter and whether or no acid were present. It should be mentioned in this connection that Kastle and Loevenhart found that the extract they used lost its activity to a very great extent on mere filtration through paper.

Apparently, acids do not act merely by liberating the enzyme. Several experiments have been made in which the material free from fat was digested, at the temperature at which the hydrolysis is ordinarily effected, with the amount of sulphuric acid in presence of which hydrolysis of fatty oil takes place rapidly; when washed free from acid, the product was incapable of effecting hydrolysis whether used alone or together with fresh acid. Evidently the enzyme had been destroyed.

The Ricinus enzyme has been found to have but little action not only on ethylic butyrate, on acetin and on dimethylic tartrate and racemate, but also on ethylic mandelate, which, according to Dakin, is readily attacked by animal lipase.

It is difficult to resist the impression that the differences observed are not merely consequences of differences in stability of the various ethereal salts but that the Ricinus enzyme is possessed of properties which make it specifically capable of promoting the hydrolysis of glycerides of the higher fatty acids. And in view of recent observations on the action of so-called co-ferments, the part which acids play in promoting hydrolysis is specially interesting.